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INFLUENZA LABORATORY TESTING IN DELAWARE: SIGNIFICANT CONTRIBUTION TOWARDS THE INFLUENZA VACCINE RECOMMENDATIONS

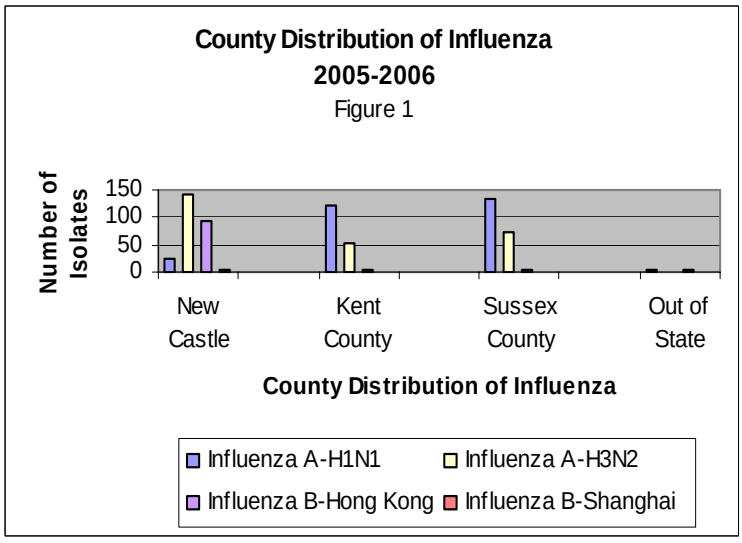
By Jong-ho Jean, Ph.D.; Susan Dee, BA; Jill Walters, MLT; Mary Ann Brown, MT, SM; Anna Linz, BS

The influenza surveillance season for the year 2005-2006 officially ended this past May. We would like to take this opportunity to share with you the laboratory activities and our plans for the new season.

The volume of requests for influenza testing has increased continually each year since 2001 when we set a goal to expand our diagnostic testing services. Even though the rapid influenza test incentive was not available this year, we received the highest volume of specimens ever for influenza testing. As shown in Table 1, total specimens submitted to the Delaware Public Health Lab increased from 134 in 2001 to 2097 in 2006.

According to the final report from the Centers for Disease Control and Prevention (CDC) for the 2005-06 season, 135,973 specimens were tested for influenza with 17,068 (12.6%) being positive. Of the 13,857 positives that have been subtyped 5,228 (92.6%) were influenza A (H3N2) and only 420 (7.4%) were influenza A (H1N1).

In Delaware, among the 2,097 specimens tested, 661 (31.0%) were positive, including 550 influenza A and 111 influenza B viral isolates. Interestingly, among the 550 A isolates, 281 (51.1%) were A (H1N1) and 269 (48.9%) were A (H3N2). The total number of influenza A (H1N1) isolates in Delaware was more than that from the rest of the nation added all together, according to Dr. Alexander Klimov from the World Health Organization (WHO) Collaborating Center for Influenza at the CDC. Furthermore, 91.7%



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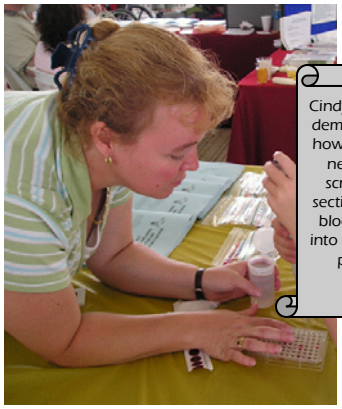
Inside this issue:

A FAIR DAY FOR PUBLIC HEALTH

Four staff from Delaware Public Health Lab attended the Family Health Fair at the Delaware State Fair on July 25, 2006. Cindy Pearson, John Caldwell, Anna Linz and Foday Turay staffed a multi-table exhibit demonstrating the FUN in laboratory science to many children and adults who attended the fair this year. Children enjoyed the hands-on color changing experiments to test the pH of drinking water, as well as the Newborn Screening spot distribution and pipetting station. Presentations also included fluorescing positive drinking water, newborn screening display and coloring pages, and samples of preserved bats, brains, hummingbirds and an Ascaris worm that fascinated some and repelled others. Judging from the degree of participation by visitors to the booth, our goal of increasing the awareness of Public Health activities was achieved.

Anna Linz, John Caldwell and Foday Turay were educating spectators of all ages.

John Caldwell is demonstrating how to identify fluoride in drinking water.



Cindy Pearson demonstrates how the lab's newborn screening section drops blood spots into microtiter plates.

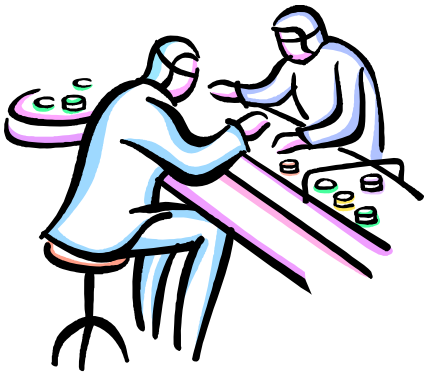
EMPLOYEE GROUP OF THE QUARTER

The Microbiology Section was nominated as the employee group of the quarter for working effectively under extreme staffing shortages . This group of dedicated conscientious microbiologists has worked and continues to work under staff shortages during an extended period of time still maintaining quality and timely results. Normally the microbiology section consists of the lab manager, a microbiologist III and six microbiologist II's. This section provides quality microbiological test results for numerous providers throughout the state for sexually transmitted diseases (STDs), tuberculosis, bioterrorism, serotyping and culture- based tests. Annual testing volumes exceed 100,000 specimens for this section. From December through June 2006, this section has had multiple staff out for sick leave, vacation, scheduled training, jury duty and disability. There was a four week time period where 3 technologists were out for various reasons and everyone pitched in to help in all of the areas, staying late and working extra weekends to get the work done. Special thanks go to Gaile McLaughlin, Donna Colatrella and Debbie DeRocili for all their help and for going above and beyond to get things done. There was an outbreak in the high school wrestlers at this time which added even more workload of cultures to this section as well as our continuous rollout and training of state service centers in our new laboratory information management system (LIMS) application. To add a little more to the madness, techs trying to keep all the specimens straight in two computer systems where one third of those are in LIMS and the rest are temporarily in another STD application. Workload for the first quarter of 2006 had doubled the workload from the same quarter 2005 for STD specimens. This section has exceptional employees that work extremely well together even under stressful situations and can still maintain a sense of humor and complete the work.

NEW GENPROBE METHOD FOR STD TESTING

By Debbie Rutledge, BS

During the lazy days of summer, the Delaware Public Health Lab microbiology laboratory section has been extremely busy training and validating a new method for Chlamydia and Gonorrhea nucleic acid amplification. The lab switched to the Genprobe Aptima Combo 2 Assay on July 10, 2006. This switch was complicated because it is during this same time of year that the Division's fiscal offices annually "shut down" as all the financial accounts for the fiscal year are closed out and finalized. This process makes it a difficult period of time to purchase supplies. It is also a difficult time to train our customers on the new collection procedures. Most schools and colleges are closed during the summer and many agencies have minimal staffing due to scheduled vacations. Furthermore, the lab must continue to perform the daily work with staff vacations scheduled and at the same time learning and validating the new method. Despite these challenges, the microbiology staff completed the conversion successfully.



The Aptima Combo 2 Assay is a target capture nucleic acid probe test for the in vitro qualitative detection of ribosomal RNA (r-RNA) from *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (GC). Combining the advanced technologies of transcription-mediated amplification (TMA) and dual kinetic assay (DKA) to test for these organisms from a single specimen, the Aptima method detects Chlamydia and GC in both symptomatic and asymptomatic males and females from endocervical, urethral, and urine specimens.

This Genprobe method offers significant advantages: the highest sensitivity available with urine and swab sensitivity being equivalent; high specificity for both male and female urine and swab specimens; efficient specimen processing; and superior specimen transport stability. This method is the only nucleic acid amplification test (NAAT) that amplifies RNA. At least 2000 copies of r-RNA are present in each bacterium (in contrast to only a few copies of the DNA targets), thereby improving sensitivity. A target capture step binds primer-bound nucleic acid targets to a magnet prior to amplification, allowing substrate inhibitors to be cleansed from the sample improving sensitivity. This method has minimal problems with false positives due to cross-reactivity with genes from similar organisms primarily other Neisseria species.

Sexually transmitted diseases (STDs) due to Chlamydia and Gonorrhea have been a focus for Public Health for many years. In 1993, CDC recognized that untreated infections can lead to complications, including pelvic inflammatory disease (PID), ectopic pregnancy, infertility and chronic pelvic pain. At that time, the Infertility Prevention Project was initiated by CDC and regional project areas were created to focus on addressing this national problem. Across the United States, these infections are primarily found in the age range of 14-25 year olds. In Delaware, the prevalence rate for infection in this age group is around 9% for Chlamydia and 2% for gonorrhea. Our lab has an annual testing volume of 25,000 specimens from state clinics, school-based wellness centers, juvenile detention centers, correctional facilities, universities, planned parenthood and community based centers.

By changing to Genprobe's Aptima method, the laboratory has improved the quality of results reported. Longer specimen stability allows for fewer specimens being rejected due to transport time constraints. For the patient, this can mean more accurate diagnosis and timely treatment which can eliminate the spread of disease and prevent future complications.

References:

Gen-Probe Aptima Combo II Package Insert, 2005-11., IN0037-06 Rev.A., Genprobe Incorporated.

Renault,C.A.,Hall,C.,Kent,C.K., and Klausner, J.D. July 2006. Use of NAATs for STD diagnosis of GC and CT in non-FDA-cleared anatomic specimens. MLO 12-22.

AVIAN INFLUENZA: PANDEMIC POSSIBILITY

By Rebekah Parsons, BS

Humans are susceptible to infection from three different types of influenza viruses—types A, B and C. While type C causes only mild respiratory illness and is stable enough to be excluded as a pandemic threat, types A and B can cause more severe illnesses. Continual viral mutation creates a greater pandemic potential. Historically, influenza type B has been associated with less severe epidemics than influenza type A. Influenza B is primarily a human virus and therefore, less likely to be a deadly threat. Influenza A, however is mainly a virus circulated among wild birds, but can also infect humans, pigs, horses, and many other common animals thus creating a pandemic possibility.

Influenza A can be broken down further into subtypes based on two proteins found on the virus surface. Hemagglutinin (HA) forms 15 subtypes with H1, H3, H5, H7, and H9 being the most prominent and can combine with any of the 9 subtypes of Neuraminidase (NA). The subtypes such as H1N1, H1N2, and H3N2 which are in wide circulation in the general population are commonly referred to as the "human influenza virus." The "avian influenza virus" designates the subtypes that are mainly found in birds, but can also infect humans. The influenza A subtypes H5, H7, and H9 are considered to be forms of the "avian influenza virus." The H9 subtype is only found in a low pathogenic form with very mild symptoms and has rarely infected humans. The H7 subtype exists at either a high pathogenic form or a low pathogenic form and like the H9 subtype has rarely been known to infect humans. The H5 subtype occurs in either low or high pathogenic form and includes the H5N1 virus which has been the main focus of concern. H5N1 is highly contagious among birds and creates a risk in people who have been exposed to secretions or excretions (saliva, nasal secretions, or feces) from infected birds or had direct contact with the birds. Although wild birds act as natural reservoirs for influenza A, they can act simply as carriers showing no signs of illness when infected. The domestic bird and poultry population are at much greater risk, reaching a mortality rate of 90-100% within 48 hours of infection. Human infection with H5N1 is considered to be rare. However, this virus subtype has caused the largest number of cases of severe illness and death in humans with the first outbreak in Hong Kong in 1997. [1] The recent outbreak of H5N1 began in Asian countries in 2003. Since then, 247 people have been infected resulting in 144 deaths as of September 19, 2006.[2,3]

A pandemic possibility is created when the influenza virus mutates to become easily transmitted between humans with no vaccine available and little public immunity. The difficulty in preparing a vaccine is compounded by two forms of mutations which can occur in the virus: antigenic drift and antigenic shift. Antigenic drift is defined by unpredictable, spontaneous point mutations and produces minor changes to the nucleic acids in the genes which code for surface antigens. The genetic variation initiates a morphological change in the antigen so that old antibodies are no longer recognized. The generation of new virus strains is the final result. Antigenic shift which is a major, abrupt mutation, elicits the naming of a new virus subtype. This massive mutation could potentially rearrange and combine genetic sequences from different viral strains, human and avian, leading to a pandemic. Pathogenically, the main difference between human influenza and avian influenza is the cellular receptors to which the viruses are capable of binding. Currently, avian influenza viruses are unable to bind to non-ciliated bronchial epithelial cells. An antigenic shift which would make this type of binding possible could create a worldwide pandemic. The pharmaceutical industry is working on a vaccine or anti-H5N1 antiviral. The avian influenza virus has been proven to be resistant to amantadine and rimantadine; how-

ever, researchers are studying the potential effectiveness of oseltamivir and zanamavir.[3]

The Environmental and Molecular Microbiology section of the Delaware Public Health Lab (DPHL) employs protocols for identifying human respiratory viruses: influenza A, influenza B and adenovirus. Extraction methods are in place which can isolate viral RNA from influenza and viral DNA from adenovirus simultaneously. These viruses can be detected quickly using real-time polymerase chain reaction (PCR) platforms.

DPHL is capable of conducting further enhanced surveillance of Influenza A specimens.

Molecular protocols have been validated in which influenza A can be subtyped into H1, H3, H5, or H7. In addition, DPHL has implemented and is proficient in real-time PCR detection of Asian H5 influenza using sequence specific primers. In preparation for the possibility of Avian Influenza, 10 staff members have been trained and are proficient in performing real-time RT-PCR using primers specific for Avian influenza. Using methods validated on the ABI 7000 and the Cepheid Smart Cycler II, up to 50 specimens can be tested daily for Avian influenza.

Although Avian influenza is not currently transmitted from human to human, contact with infected poultry can cause human infection with a mortality rate of over 50%. With about 50 chickens for every person in Delaware, Public Health and the DPHL must be prepared. A rapid

reliable detection method such as RT-PCR is essential to provide adequate containment and fast treatment.

References

[1]Rosner, F. Avian killer flu pandemic:fact, fear, or fiction. The Israel Medical Association Journal. Jun 2006; 8(6) 371-2

[2]Wu, TZ., Huang, LM. Avian Influenza. Chang Gung Medical Journal. Nov 2005;28(11)753-7

[3]World Health Organization

Welcome

Morzo Tugultschinow has received a warm welcome from his coworkers, since July 10, 2006, the day he started to work in the Virology Section.

Morzo graduated from Ramapo College in Mahwah, New Jersey with Bachelor's degree in Biology. He went to Miami Dade Medical Center to receive his license in Medical Technology Certify in Florida as a medical Technologist in hematology, chemistry, serology, immunology and microbiology. He is licensed by ASCP as a medical laboratory technician. He worked for ZLB Plasma Services Company, Florida for several years and with good experience and background in performing general biological / biochemical testing. Morzo also had experience in testing HIV-1/HIV-2, HBV and HCV, etc. Morzo had teaching experience in the pas and he taught students with various classes from kinder garden to 6th grade.

With great attitude and hard work, smiling and always wanting to learn more and to do more, Morzo has won respect from his coworkers.

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Influenza Laboratory Testing in Delaware, *continued*

of A (H1N1) isolates were from Kent and Sussex counties in the central and southern parts of Delaware and only 8.3% of A (H1N1) isolates were from New Castle County in the northern part of the state (Figure 1).

Fifty of 281 A (H1N1) isolates from Delaware were submitted to the Influenza Laboratory at the CDC for further analysis. According to CDC, except for one isolate which showed a double infection with A H1 and H3, our isolates are all related antigenically to A/New Caledonia/20/99 which was the World Health Organization recommended H1 component of the 2005-2006 vaccine for Northern Hemisphere, 2006 Southern Hemisphere and 2006-2007 Northern Hemisphere.

Each year, DPHL influenza strain identification data are submitted to CDC. These data are combined with data from laboratories around the world for use by WHO in making recommendations for formulating vaccine for the coming years. Because of the unique findings of Delaware's influenza surveillance program during the 2005-2006 season, our subtyping data will be particularly important in formulating those recommendations.

It is also noteworthy (Figure 1) that no influenza B-Shanghai virus was isolated from Sussex County and the majority of influenza B-Hong Kong was isolated from New Castle County in the 2005-2006 season.

The accepted "Gold Standard" for isolating and identifying influenza in the laboratory is cell culture. Cell cultures must be incubated long enough to detect cytopathic effect (CPE) before identification procedures can be performed. Rapid direct antigen detection tests are available at many clinical laboratories, but their sensitivity and specificity is not optimal for the public health laboratory.

For the upcoming season, a new rapid culture method, the shell vial assay will be implemented. This assay will shorten the time to detect and identify virus in clinical material. Cell monolayers are grown on coverslips in flat-bottomed vials. After inoculating several vials with a specimen, the vial is centrifuged to enhance viral infection of the cells. Viral antigens can be identified in the early stages of replication, before CPE is detectable.

Shell vials can be "blind tested" with immunofluorescent screening reagents at 24 hours post infection. The respiratory pool screen can test for a battery of respiratory viruses including influenza A and B. If the screen is positive, cells from the other vials can be tested with specific reagents to identify the virus. Negative cultures can be signed out in three days; positive cultures for respiratory viruses other than influenza A and B can be signed out in 48 hours. In order to subtype influenza A and B another 3-5 days may be necessary.

TABLE 1

Year	Influenza A	Influenza B	Total Positive Influenza	Total Specimens Tested
2000-2001	23	27	50	134
2001-2002	119	32	151	345
2002-2003	86	33	119	335
2003-2004	422	1	423	770
2004-2005	533	286	819	1582
2005-2006	550	111	661	2097

WEST NILE VIRUS LABORATORY TESTING IN DELAWARE: GAINING KNOWLEDGE THROUGH EXPERIENCE

By Jong-ho Jean, Ph.D.; Susan Dee, BA; Jill Walters, MLT; Mary Ann Brown, MT, SM; Anna Linz, BS

West Nile virus (WNV) emerged in the New York City Metropolitan area in 1999 and from there the virus spread across the country. Migratory birds introduced the virus in Delaware in 2000.

Since 1981, as part of Delaware's arbovirus surveillance program, the Public Health Laboratory has used both serological testing and virus isolation to identify arbovirus infection in the state. This program, a collaboration between the Department of Natural Resources and Environmental Control (DNREC) and the Department of Agriculture, provided a sense of readiness for WNV arrival. On October 19, 2000, a great-horned owl was the first identified as having a current infection with WNV in Delaware. Since then, the Delaware Public Health Lab (DPHL) has developed and implemented protocols that combine both new and conventional techniques for laboratory diagnosis of WNV infection. The U.S. Centers for Disease Control and Prevention (CDC), through the grant for Epidemiology and Laboratory Capacity (ELC) for infectious diseases, has provided funding and technical support for diagnostic testing, prevention and control measures all over the U. S.

Initially, techniques for the detection of arboviral antibodies were the hemagglutination inhibition (HAI) test, plaque reduc-

HUMAN SERUM SPECIMENS: DETECTION OF WNV INFECTION STATUS
CORRELATION OF HAI ANTIBODY TITERS WITH RESULTS OF ELISA, IFA & PRNT
TABLE 1

	HAI		ELISA-WNV			
Specimens	WNV	SLE	IgM-Capture	IgG	IFA-WNV	PRNT-WNV
No.1	<1:10	<1:10	Neg	Neg	Neg	NS
No. 2	1:80	1:40	Neg	Pos	2+	40%
No. 3	1:320	1:160	Neg	Pos	4+	73%
No. 4	1:1280	1.640	Neg	Pos	4+	100%
No. 5	1:1280	1:160	Pos	Pos	4+	100%

HAI = Hemagglutination Inhibition
IFA-WNV = WNV Specific Monoclonal Immunofluorescent Antibody Assay
IgM-Capture = IgM Antibody Capture Enzyme-Linked Immunosorbent Assay
IgG-ELISA = IgG Enzyme-Linked Immunosorbent Assey
NS = Not significant
PRNT = Plaque Reduction Neutralization Test
WNV = West Nile Virus
SLE = St Louis Encephilitis Virus
Neg- = Negative
Pos = Positive
+ = Intensity of Immunoflorescence Staining

tion neutralization test (PRNT), Immunofluorescent antibody (IFA), and IgM/IgG enzyme-linked immunosorbent assays (ELISAs). More recently, polymerase chain reaction (PCR) was developed for early detection of viral nucleic acids. In order to establish an optimal protocol to test specimens of various species for WNV infection, DPHL has conducted comparative studies of different methodologies. Those studies are reviewed in the accompanying tables.

As shown in Table 1, positive WNV IgG ELISA was observed for the Specimen Numbers 2, 3, 4 and 5. WNV IgM and IgG ELISA were sensitive screening tests, but cross-reactivity with St. Louise Encephilitis (SLE) virus was a concern. To differentiate WNV infection from SLE, HAI was performed. Results of the HAI indicate that these positive specimens were true WNV infection, even though some cross-reactivity between WNV and SLE was observed. In order to compare and confirm test results of HAI and ELISA, both IFA and PRNT were also performed for these specimens. The results of IFA confirmed the test results of both HAI and ELISA for all 5 specimens. However, only specimens 4 and 5 were positive by PRNT. PRNT is recommended as the confirmatory test; nevertheless, data here indicate that specimens 2 and 3 did not have sufficient antibody activity to neutralize the WNV antigen.

Tri-State Bird Rescue Center in Delaware plays an important role in rescuing injured birds in Delaware, Pennsylvania and Maryland. In testing serum specimens drawn from wild birds of various species and from the literature, we learned that chicken WNV-capture ELISA is capable of detecting anti-WNV IgM in serum samples from naturally infected chickens; however, the test is not reliable for use with other free-ranging birds. Blood specimens of injured free-ranging birds were therefore analyzed with the HAI test and PRNT rather than IgM ELISA. Table 2 shows that the HAI test clearly differentiated positive WNV infection from SLE for 6 birds including two American Crows, one Barred Owl and three Great Horned Owls. The results also show that PRNT confirmed HAI results with the exception of the two HAI indeterminates.

Ideally, the laboratory diagnosis of a viral disease is by isolation of the virus or detection of the viral nucleic. In practice, however, both procedures have limited utility. As shown in Table 3, among 17 patients who were diagnosed with WNV infection, 16 of 17 patients tested negative by both PCR and cell culture. Nevertheless, all 16 specimens tested WNV positive by serum IgM, confirmed by CSF IgM, or by positive serum WNV HAI.

The serum specimens of patient number 1 were WNV positive by IgM ELISA and confirmed by CSF IgM, whereas the serum specimen tested negative by HAI. The IgM ELISA may specifically detect IgM earlier than HAI testing, allowing for a more timely diagnosis of WNV infection.

Only one of 17 patients (No. 17) laboratory diagnosed with WNV infection had WNV isolated and nucleic acid detected by cell culture and PCR. The patient's serum and CSF specimens both tested negative by WNV HAI and IgM ELISA. Early studies of the phases of the viremia in WNV infection have illustrated that the viremia persists for only a very short period after the onset of symptoms. We learned later that patient No. 17 was a stem cell transplant recipient, whose immune system probably delayed antibody production, allowing the viremia to persist longer without interference by WNV antibodies.

IgM ELISA is optimal for detecting antibody produced in the very early stage of WNV infection, but in most cases, early antibody production can also be detected by HAI. HAI can be effective to test many specimens from the same species or specimens from

West Nile Virus Testing in Delaware, *continued*

various species, such as chicken, wild birds, horses, or humans. Furthermore, HAI can also differentiate antibodies to WNV from SLE simultaneously.

A positive WNV IgM ELISA in a single, acute-phase specimen is presumptive evidence of infection. Studies have shown that many laboratory-confirmed WNV encephalitis patients had anti-WNV IgM detected even one year after onset. Studies also advise caution when interpreting serologic results for WNV IgM-positive patients early in the season.

IgM produced in early infection does not always possess neutralizing activity, thus, IgM ELISA may not always correlate with PRNT. Moreover, specimens that remain unconfirmed by PRNT may reflect the lack of neutralizing ability of IgM produced in the early acute phase of infection and should not be confused with a false-positive result.

PCR and cell culture are not the choice for routine diagnosis of WNV in humans, except immune-compromised patients, since the virus disappears from blood and CSF soon after the onset of symptoms or illness.

PCR and cell culture can be useful in identifying WNV in brain or various tissues of birds, horses or other animals.

To improve our ability to diagnose arbovirus infection, DPHL has adapted a newly developed test, the microsphere immunoassay (MIA). MIA reliably discriminates between infections of WNV and SLE or dengue viruses. The assay is also capable of differentiating current WNV infection from past flavivirus infections or previous flavivirus vaccines. Furthermore, MIA tests more specimens faster. This assay has been validated recently for testing human sera and CSF specimens in the DPHL.

Hemagglutination-Inhibition and Plaque Reduction Neutralization Compatibility in the Confirmation of WNV Infection Status

TABLE 2

ID #	Bird Species	Antibody Titers of HAI to :		PRNT
		WNV	SLE	
1638	American Crow	1:5120	1:640	POS
1678	American Crow	1:10240	1:320	POS
1146	American Crow	1:1280	NT	POS
1586	Barred Owl	1:5120	1:640	POS
1907	Great Horned Owl	<1:10	NT	NEG
1698	Great Horned Owl	>1:40	NT	POS
1863	Great Horned Owl	1:2560	1:160	POS
1637	Great Horned Owl	1:2560	1:320	POS
1674	Great Horned Owl	1:10240	1:2560	POS
1784	Red Shouldered Hawk	Indeterminate	NT	POS
1805	Red Tailed Hawk	Indeterminate	NT	NEG
1725	Red Tailed Hawk	>1:40	NT	POS
52 *	Red Tailed Hawk	1:40 ; 1:160	NT	POS

HAI = Hemagglutination Inhibition
PRNT = Plaque Reduction Neutralization Test
WNV = West Nile Virus
SLE = St. Louis Encephalitis Virus
NT = Not Tested
POS = Postive
NEG = Negative
* = Both acute (1:40) and convelescent (1:160) specimens were tested by HAI
The acute specimen was QNS for PRNT.

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